Aria 4-Laser Start-Up / Shut- down for Customer Operated Sort:

During the week, the FlowCore (FC) will do the set-up for the day. However, if you will be sorting on weekends or overnight (after we have performed the shut down) you will need to start up the instrument and set it up for your sort.

1. Turn Flow Cytometer on by pressing the GREEN button on the left side of instrument.
2. Turn on Water bath.
3. Check sheath and waste levels. If filling sheath, spray ethanol on table and set sheath lid on clean surface.
4. Check the fluidics line and the air line to make sure they are attached to the Sheath Tank, not the Ethanol Tank
5. Turn on the computer and log into Windows.
    a. Account: Admin
    b. Password: BDIS.
6. Open Diva Software and log in under Admin- NO PASSWORD NEEDED.
7. Perform a Fluidics Start-up found under the Cytometer dropdown menu; follow the Wizard instructions and check them off as they pop up and you have completed them.
8. Choose the appropriate instrument config (This will open a new window, CST software. After changing config you will be closing and going back to Diva)
    a. Cytometer--View Configuration. Select appropriate config
        - 70 um
        - 130 um
        - 100 um (30PSI)
        - 100 um (20PSI)
        - 85 um

    b. DON'T FORGET TO "SET CONFIGURATION" then hit OK-->OK
    c. Close Cytometer Setup and Tracking Window
    d. Select USE CST Settings
9. Lay nozzle on kimwipe and spray with water. Dab try with Kimwipe.
10. Insert the Correct Nozzle for your sort with the red seal pointing up.

11. Open Sort Chamber
12. Start the stream and allow it to stabilize (this could take several minutes). A clean nozzle will be at or near the previously set positions (Drop 1 and Gap in the droplet monitor window). Drop down menu View→Sorting.
13. If the stream looks a little off give the nozzle a very gentle wiggle. Sometimes a weird looking stream is not clogged, it's just not seated properly. If you are using the same nozzle as the person before, the stream should be hitting the center of the collection trough. If using a different nozzle, you will probably have to adjust the positioning of the waste trough. See pictures
14. Spray waste collection trough with H20 to clear salt buildup.
15. Close Sort Chamber and instrument hood

_____OPTICAL ALIGNMENT_____

16. Run URFP QC beads (black cap vial in small fridge, Catalogue #URFP -30-2) using the URFP QC Experiment corresponding to nozzle size, record a data file and name it today's date. Add 1 drop of URFP to 1000 uL of DI H2O.

OR

17. **(CS&T)**
    a. Cytometer-->CST-->Wait for it to connect
    b. Load CST beads (1 drop in 1 ml H20)
    c. Confirm appropriate Lot number. (From beads bottle)
       Setup Control→ Setup beads→ Lot ID
    d. Select Run of "Check Performance"
    e. Follow prompt
    f. After completed close CST and select "Use CST Settings"

_____SIDE STREAMS/SORTING CALIBRATION_____

18. Re-check the stream position and appearance - if everything is still ok set "Sweet Spot" on the Droplet Monitor Window.
19. Turn on Voltage and "test sort" in side streams window
20. Set Half Left Sort stream to about 30
21. Sweep side streams laser to ensure side stream has brightest intercept (large sweep is easiest, back and forth maybe 6 to 10 times.
22. Turn off side streams (Test sort)
23. Run Accudrop Beads (blue cap vial in small fridge, Catalogue #345249) under Accudrop Drop Delay Experiment. Load Tube, Acquire,Activate Optical Filter button on Sort Stream Window and and beads should display 100% in the waste stream. Activate voltage button on Sort Stream Window. Press the Sort button in the Sort Layout (Under Global worksheet-->Global Sheet 1→ Sort Layout) , making sure the deflection of the inside left stream is inside the second box. Adjust flow rate so that threshold rate is about 1500. Press Autodelay button on Sort Stream Window. Follow wizard instructions as they pop up. Choose Start Run and the Auto Drop Delay function should begin to plot a curve to find the best drop delay value.
24. Don't forget to run Ethanol prior to your samples clean out beads etc...
25. Log out of Admin and log into "User"- NO PASSWORD
26. You are now ready to set up plots and gates in your own experiment!! ☺

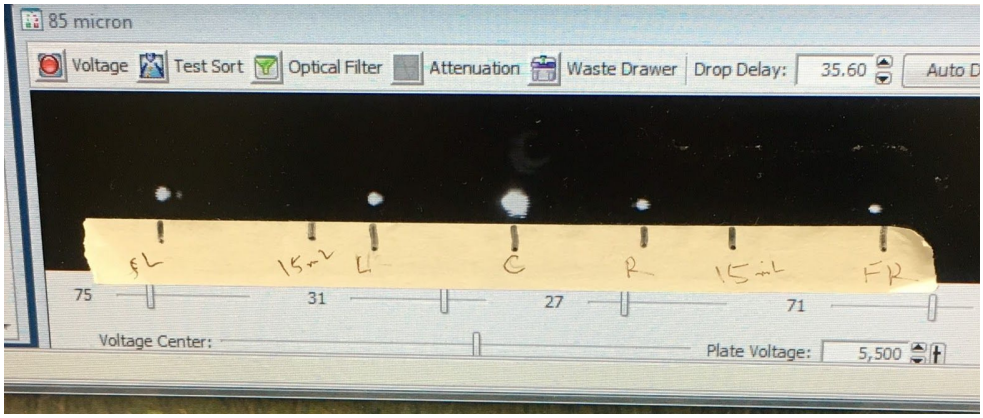
<u>How to adjust the side stream lasers (perform before Accudrop)</u>

Turn on side stream voltages
Turn on Test Sort
Sweep laser until side stream and center stream are as bright as possible

<u>How to adjust side streams for each collection tube (5ml vs 15ml)</u>

Using the slider bars, adjust stream positions to match the appropriate collection tube. (The example above shows streams set for collection in 5 mL polypropylene collection tubes. Streams should be aligned with tape on the monitor.

If collecting using 15 mL collection tube, have near right and near left streams aligned with 15 mL mark on alignment tape.



How to add gates/ stop counts/precision mode to sort layout

Insert new sort layout under “sort” menu
Select appropriate number of populations (2tube, 4 tube, 96well plate) etc
Select “New Purity” under Precision. Or “Single cell” for 96well plate
Right click and Add a population to each column
Hightlight column and set “Target events”=stop counts for each tube

How to run Accudrop (make sure streams laser is optimized prior to this step)

Open Accudrop experiment
Open sort layout window (Make sure “Fine Tune” is selected for “Precision” mode
Add one drop of accudrop to 1ml of H20
Adjust flow rate to ~1,500events/sec
Select “Auto delay” from Streams window.
Select “sort” and then “cancel in window that pops up.

How to Backflush between samples

Cytometer menu⟶ Cleaning modes-->Sample line Backflush-->Start

85um tip= 425,000 cells/ml in collection tube

=with 1ml of collection media you can fit about 1.5million in 5ml tube

= with 2ml of collection media you can fit about 5 million cells in 15ml tube

70um tip= 1million cells/ml in collection tube

= with 1ml of collection media you can fit about 35million in 5ml tube
= with 2ml of collection media you can fit about 12million cells in 15ml tube

FACS ARIA INSTRUMENT
Shut Down Procedure

- Turn the stream off.
- Take out the nozzle and put away.
- Insert the Closed Loop Nozzle.
- Run Flow Cell Clean 1x using Contrad as solution.
  Cytometer-->Cleaning Modes→ Clean Flow Cell
- Run Flow Cell Clean 2x using DiH20 as solution.
- Turn Machine Off (GREEN BUTTON)
- Release pressure from Sheath Tank
- Exit out of Diva Software.